Cell Therapy for Multiple Sclerosis

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Introduction

Multiple sclerosis (MS) is an autoimmune, inflammatory demyelinating condition. T helper cells and antigen presenting cells ultimately coordinate the production of inflammatory cytokines and chemokines within the central nervous system (CNS) parenchyma, leading to recruitment of pro-inflammatory mediators, and finally destruction of the myelin sheath and axons (1). Axonal loss presents a key pathophysiological mechanism of progressive disease; and progressive axonal damage is likely to be due to a combination of persistent myelin and oligodendrocyte loss (causing loss or trophic support and sustained demyelination-induced conduction block) and continuous exposure to injurious agents (2).

MS affects some 25 million people worldwide. More than 80% of patients ultimately develop progressive disability, despite commencing with a relapsing remitting course, with a median time to progression of 15 years (3). 10-20% of patients have a primary progressive (PPMS) course (4). The costs of MS increase dramatically with increasing disability and impairment (5). In stark contrast to relapsing-remitting disease, for which there is a wide and still increasing choice of drugs, there are no conventional treatments that offer significant efficacy in preventing or reversing the accumulation of disability (6). As with so many other neurodegenerative conditions, cell therapy for MS appears to be a highly attractive therapeutic option and, over the last few decades, there has been rapid translation from *in vitro* and *in vivo* experimental studies to safety and feasibility trials across the world. Nonetheless, the challenges facing the development of cell therapy for the treatment of MS remain daunting.

MS is generally accepted to be an autoimmune disease, with oligodendrocytes and the myelin sheaths they synthesise and support representing the primary target of this autoimmunity, although much remains extremely poorly understood. Not least, we do not know what triggers the disease. We also do not know why inflammation occurs in patches in the CNS, rather than diffusely, nor why some areas of the brain and spinal cord are more susceptible than others. And perhaps least of all do we understand how this patchy inflammatory demyelination relates to the progressive neuronal and axon loss that underlies the progressive disability occurring in most patients with MS, a phase of the disease which has the pace and features far more suggestive of a degenerative than an inflammatory condition. These areas of significant uncertainty clearly impede the development of rational therapies, cell-based and otherwise. Another challenge is the lack of clinically relevant experimental models of disease – experimental autoimmune encephalomyelitis (EAE) models are usually characterised by relapses with rapid recovery of inflammatory damage but

no progressive neurodegenerative phase and models of focal, chemically-induced demyelination demonstrate little or no inflammatory toxicity. Assessing neuroprotective therapies in MS also presents a continuing major challenge because of the variability of disease features and course of the disease, combined with the insensitivity of generic clinical outcome measures (7).

The complexity of the disease helps to explain the complexity of current approaches to cell therapy in MS. There are three quite different types of cell therapy actively being explored, variably aiming to exploit the therapeutic properties of different stem cells to achieve inhibition of the immune pathogenesis of disease, neuroprotection and to promote repair. This review will present an overview of where we are now with cell therapy in MS.

1. Approaches to cell therapy in MS

Replacing oligodendrocytes

In 1977, it was shown that exogenous myelinating cells injected into demyelinated lesions in the rodent CNS achieved successful remyelination (8). Transplantation of myelin forming cells, either directly into MRI-disclosed lesions, or with the intention of their dissemination through the entire neuro-axis, has been a major aim ever since (9). In a variety of experimental paradigms, many types of transplanted cells have successfully remyelinated acute focal demyelinated lesions in the adult CNS (10).

Embryonic stem cells were until recently considered the best putative candidates for such an approach. However, it is now clear that human dermal fibroblasts, and other somatic cells, can be reprogrammed to pluripotency via retroviral transduction (induced pluripotent stem cells or iPSCs); and more recently the same has been achieved by chemical or pharmacological approaches. MS-patient derived iPSCs can differentiate into oligodendrocytes (as well as astrocytes and neurons) with normal karyotypes, and these can then achieve myelination *in vivo* in the *shiverer* mouse (11). IPSCs are probably now the more favoured cell vehicle for oligodendrocyte replacement, although the protocol for induction is inefficient, and concerns remain about genomic stability and the tumour risk associated with using these cells therapeutically. (12).

In addition, however, there are conceptual difficulties with this approach. Both oligodendrocyte progenitors and neural precursors are in fact present in significant numbers in MS lesions, yet they are unable to regenerate myelin, perhaps as they are unable to differentiate, and show arrested development (3). It is not clear that adding more cells would help under these circumstances. Also, while inflammatory demyelinating lesions cause relapse-related neurological dysfunction, their direct relationship to chronic progressive disability is unclear and uncertain; neither lesion load, lesion site, nor the number of relapses correlate well with chronic disability (3). It has therefore become difficult to see how patients with secondary and primary progressive disease might benefit from directly injecting oligodendrocyte progenitors into MRI-disclosed lesions (3) – though a case might still be made in occasional patients with very large lesions causing relapse-related symptoms, who develop disability as a direct effect of significantly incomplete spontaneous remyelination.

What is undeniable however is that the intensive study of the molecular and cellular neurobiology of myelin repair stimulated by and originally directed towards oligodendrocyte replacement therapy has yielded invaluable new knowledge concerning remyelination, knowledge which has directly lead to molecular candidates for promoting myelin repair – either small molecules as conventional pharmacological agents, or monoclonal antibodies, several of which are now undergoing early phase clinical trials (13).

Autologous haematopoietic stem cell transplantation

Autologous haematopoietic stem cell transplantation (AHSCT) is a promising treatment for MS, perhaps particularly for those who have not responded to conventional immune therapies (14). AHSCT is a well-established procedure for the treatment of poor prognosis haematological malignancies, and in the last 20 years it has been explored to treat patients with severe autoimmune diseases who were deteriorating despite receiving standard treatments (15). The rationale for this approach is that ablation of the aberrant immune system followed by reconstitution of a 'new' immune system from haematopoietic stem cells should substantially alter the characteristics of the T-cell responses, and other immune reactivities, and so potentially improve the clinical course of autoimmunity, including MS (16). Following early reports such as that from Fassas *et al* (17), MS has become one of the most common autoimmune diseases to be treated with AHSCT (18). In 1997, the Autoimmune Diseases Working Directive (ADWP) of the EBMT set guidelines for application of AHSCT to autoimmune disease, and advised that all cases treated should be registered within the European Group for Blood and Marrow Transplantation (EBMT) database (19). Over 2000 patients with an autoimmune disorder have now been reported to the Registry of the EBMT as having been so treated, of whom more than 800 have MS.

The source of stem cells is commonly bone marrow, cord blood or peripheral blood. Peripheral blood stem cells (PBSC) contain more progenitor cells and mature lymphocytes than bone marrow. In addition, with PBSC, there is the ease of collection, since bone marrow has to be collected by a general anaesthetic. However, since the numbers of PBSC are small, they must first be mobilised from the bone marrow using cyclophosphamide (Cy) or growth factors such as granulocyte colony stimulating factor (G-CSF). The combination of Cy and G-CSF is generally preferred as Cy reduces the potential risk of MS exacerbation in response of G-CSF and the inclusion of Cy in the mobilisation regime decreases the number of T cells in the apheresis collection (20).

Once harvested, haematopoietic stem cells (HSCs) can be manipulated (termed *in vitro* purging) by either CD34+ positive selection for lymphocyte depletion and/or directly purged with anti-lymphocyte antibodies (such as with CAMPATH 1H or cytotoxic agents) (21). HSCs carry the CD34 and Thy-1 markers and these surface markers are usually used to isolate cells including early progenitors (21).

Having collected and prepared HSCs for transfusion, the patient's own immune system must be ablated, or at least suppressed sufficiently to allow the infused HSCs to regenerate the immune system in preference to the 'original' immune system re-asserting itself. This process is 'conditioning'; different conditioning regimens can be administered before the infusion of CD34+ autologous cells (22), and the patient is usually admitted for conditioning. Common regimes utilised vary in intensity; examples are listed below:-

- high intensity regimes include total body irradiation (TBI) or high dose busulfan
- low intensity conditioning regimens utilise cyclophosphamide alone, melphalan alone, or fludarabine-based regimens
- intermediate intensity regimens include other combinations such as BEAM, or anti-thymocyte globulin (ATG) and cyclophosphamide (23)

The combined carmustine (**B**iCNU®), **E**toposide, cytarabine (**A**raC) and **M**elphalan (BEAM) conditioning regime is considered the most effective (16). The risk of transplant-related mortality (TRM) in HSCT, defined as deaths occurring in the first 100 days (24) has decreased from 2001, according to EBMT, likely due at least in part to the avoidance of aggressive regimes which resulted in toxicity, such as the use of busulfan (20).

Finally, following the conditioning stage, at least $2x10^6$ CD34+ positive cells/kg of body weight is required for haematological reconstitution (21). Haematological recovery requires a mean of 12 days to reach a neutrophil count >500/µl, and 10 days to reach a platelet count of $>20x10^9$ (22).

HSCT also has been shown to normalise microRNA and gene expression and improve the immune-regulatory network (25). Using microarray DNA-chip technology, AHSCT was found to alter gene expression of peripheral CD4+ and CD8+ T-cell subsets – clusters of reconstituted CD8+ T cells of MS patients two years after transplantation were more similar to healthy controls. There were more extensive changes in the expression of genes involved in the effector immune response (26). AHSCT induces profound modifications in the immune-regulatory compartment, such as a transient increase in regulatory FoxP3+ T-cells (22). In MS, AHSCT renews the CD4+ repertoire, blunts the encephalitogenic effector response by reducing Tc17 and Th17 peripheral blood T-cells, impairs antigen presentation, and increases the numbers of immune-regulatory cells (22).

Conversely, autopsy material from five MS patients who received AHSCT showed that there was ongoing evidence of active demyelination, while the inflammatory infiltrate within the lesions showed predominantly CD8+ cytotoxic T cells, with high numbers of acutely damaged axons. This implies that despite AHSCT (and the accompanying immunosuppression), there is ongoing disease activity – arguably also reflected in patients exhibiting continued disease progression and/or MRI activity in AHSCT trials (27). AHSCT has also been associated with rapid brain volume loss in the months subsequent to treatment, which then declines after two years. The initial loss may be due to pre-transplant disease activity or result of the intense immune-ablative conditioning procedure (28).

Nonetheless, it seems clear that AHSCT can reduce clinical relapse activity dramatically, with a potency comparable (or, it has been claimed, superior) to the current most powerful licensed therapies, alemtuzumab and natalizumab. Its morbidity and mortality may, however, be greater, and so the place of AHSCT in the overall treatment paradigm of relapsing remitting MS remains to be defined. Comparative studies are required.

Mesenchymal stromal cells (MSCs) and related cells

As well as haematopoietic stem cells, bone marrow contains other cell types with stem cell-like properties, including mesenchymal stromal cells. Many stem cell researchers concentrate on these cells, not in the least because of their

properties in promoting cell repair through multiple mechanisms, combined with immune-modulating and immune-suppressive actions. MSCs can stimulate local proliferation of endogenous neural precursors, secrete various trophic factors, and protective antioxidants such as superoxide dismustase-3, reduce gliotic scar formation and promote CNS neurite outgrowth and remodelling (3, 29, 30).

MSCs are a rare and heterogeneous population of cells, which are relatively easy to extract and expand from a number of tissues in the body including bone marrow. They were first described by Friedenstein *et al* in 1968 (31). No single marker or even combination of markers specifically identifies MSCs. Criteria proposed by the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy include plastic-adherence during *in vitro* expansion, absence of hematopoietic surface markers (such as CD45, CD34), presence of CD73, CD90, and CD105 surface markers, and ability to undergo *in vitro* differentiation into adipocytes, chondroblasts and osteoblasts (32). Their normal function is to support HSCs within the bone marrow niche, but they also have a systemic role, following release into the circulation, in maintaining vascular and immunological homeostasis and facilitating tissue repair (33). They have a selective ability to home to sites of tissue damage or inflammation, a process mediated by chemokine receptors and other adhesion molecules (1). MSCs have a number of immuno-modulatory properties, such as suppression of T cells leading to a concomitant increase in the Th2 cytokine IL4 (34). MSCs can promote self-tolerance by inhibiting the ability of dendritic cells to become antigen presenting cells (12).

MSCs also have a number of neuroprotective properties. They promote oligodendrogliogenesis, neural survival and neurite outgrowth, and protect neurons against oxidative stress, partly through the secretion of neurotrophins such as brain derived neurotrophic factor and nerve growth factor (29, 35). Rather remarkably, they can also protect tissue by directly transferring mitochondria to vulnerable cells through a process involving membrane fusion (36). They can fuse with cells to promote target cell survival (37).

MSCs therefore offer potential therapeutic benefit in MS by restricting inflammation, protecting axons, neurons and glia, and promoting remyelination (38). Systemic transplantation of autologous or allogeneic MSCs in relapsing-remitting or progressive models of EAE showed a decrease in T and B cell responses, accompanied by clinical and histological improvements, reducing the number of inflammatory lesions, reducing axonal loss and preserving myelin structure (39, 40). Immunological analysis revealed an increase in the proportion of CD4+CD25+ regulatory T cells, a decrease in the proliferative responses of lymphocytes, and the expression of CD40+, CD83+, CD86+ and HLA-DR on myeloid dendritic cells at 24 hours after MSC transplantation (41).

There are, however, a number of theoretic risks in the application of MSCs. Close monitoring during infusion is necessary because of potential toxicity related to an allergic reaction to foetal bovine serum (FBS) in the culture medium, dimethyl sulfoxide (DMSO) in the freezing medium, and the risk of pulmonary embolic phenomena. Utilisation of FBS raises several issues: for example, anti-FBS antibodies might react with FBS antigens adherent to MSCs, leading to rejection or to infusion-related allergic reactions. FBS could also theoretically transmit infection, including zoonoses such as bovine spongiform encephalopathy (42, 43). Therefore, the development of serum-free culture methods is a priority. Culture-expanded MSCs can trigger a so-named 'Instant Blood-Mediated Inflammatory Reaction' (IBMIR), mediated by the innate immune system (33, 43, 44). A further infection risk comes with *ex-vivo* expansion; which also may enhance the possibility of ectopic tissue formation. When culture-expanded MSCs were administered

intraventricularly, they migrated into the brain parenchyma and formed cellular masses with focal inflammation. Local tissue damage and collagen-fibronectin deposition were observed (45). Cancer related to malignant transformation of culture-expanded MSCs or permissive effects of immunosuppression is also a theoretical concern (33). *In vivo*, MSC transplantation could conceivably have pro- or anti-inflammatory effects in MS (46): suppressing the 'wrong' component of the immune system, or precipitating (perhaps by some allergy-related mechanism) a general increase in immune activation, could conceivably exacerbate relapsing-remitting MS. One recent report described a patient with MS who developed acute disseminated encephalomyelitis-like illness six hours after the third of three monthly intrathecal injections of autologous MSCs (47).

The optimal route and dose of MSC administration is still debated. If we assume that the cells are required to access the CNS to be clinically effective, a drawback of intravenous administration of MSCs is that cells will become trapped in the lungs, or will home to lymph nodes and other tissues, reducing the number of cells available to migrate to the CNS (41). An intrathecal approach for cell-based therapies in neurological disease such as MS, in which areas of tissue damage are widespread throughout the neuro-axis, may increase the likelihood of migration of the injected cells to the closer proximity of areas of CNS damage. The injected cells may circulate with the flow of cerebrospinal fluid and so gain a better chance of reaching affected areas (41); but intrathecal delivery of MSCs is, however, complicated by a common meningeal reaction. Very little evidence is available on formal dosing of MSCs for transplantation; a commonly used dosage is $1\text{-}2x10^6$ cells per kg (33).

The extent of engraftment and duration of survival of donor MSCs after transplantation in humans is largely unknown. Autopsies of 18 patients who received HLA-mismatched MSCs for complications of HSCT, showed little evidence of MSC DNA in donor tissue (48). Engraftment and magnitude of therapeutic response correlate poorly, and a paracrine effect with persistent therapeutic benefit that is not dependent on surviving implanted cells is postulated for some treatment effects – the so-called "hit and run" mechanism of action. While sustained beyond the duration of cell 'residence', such effects are likely ultimately to subside, and so repeated administration may be required. Harris *et al* found that multiple administration of MSCs in a rodent inflammatory demyelination model was more likely to help arrest progression (49). The risk of sensitisation would, however, likely confine such an approach to autologous MSCs (33) – repeated administration of allogeneic MSCs does generate problematic immune reactivity (50). The possibility that recurrent administration of autologous MSCs may be required raises further practical questions – would these be achieved by repeated harvests, or perhaps through expansion with cryopreservation? Before culture-expanded MSCs can be seen as an "off-the-shelf" product (33), comprehensive certification of the donor would be required to rule out infection and cancer. Regulatory hurdles would be more difficult.

We do not know whether autologous or allogenic MSCs might be more effective. There is a theoretical concern that autologous cells from a patient with an inflammatory and degenerative disorder may have defective immunomodulatory, tissue protective or reparative capabilities (33). This possibility has been explored by Mallam *et al* (51) and by Mazzanti *et al* (52), where MSCs from patients with secondary progressive MS (SPMS) and relapsing remitting MS (RRMS) were found to be similar to controls in a number of parameters. However, Mazzanti *et al* did find that MS patient MSCs had significantly greater lipopolysaccharide-stimulated IP10 production compared to healthy controls, while Mallam *et al* had only explored a relatively small number of patients with MS. In an interesting study, MSC gene expression

profiles and also function were compared between control patients and individuals with MS both before and then after autologous HSCT. Pre-HSCT, MSCs had distinct transcriptional profiles compared to controls, including downregulation of *TGFB1* and *HGF* genes, and reduced secretion of IL-10 and TGF-B. Six months after transplantation, the transcriptional profile remained similar to pre-transplant AHSCT; post-transplantation MS patient MSCs were closer to pre-AHSCT samples than to healthy MSCs. These findings therefore showed that MS-MSCs exhibited phenotypic changes, distinct transcriptional profiles and functional defects in immunomodulatory and immunosuppressive activity, not 'corrected' by HSCT, implying that allogeneic bone marrow MSCs might be better as a putative treatment cell type (53). The question, however, is not definitively resolved, and studies of the phenotype and function of MSCs isolated from MS patients, including those involved in ongoing and planned treatment trials, will be important to explore this issue further (33).

Human bone marrow derived MSCs can be safely extracted, expanded *in vitro* and, despite the theoretical risk, do not seem to be susceptible to malignant transformation; thus they appear to be suitable for clinical application (54). To date the largest studies of therapeutic MSC transplantation have been in haematological malignancy, breast cancer, ischaemic heart disease, and in graft-versus-host disease (55). With no induction or conditioning, trials involving MSCs have no treatment-related mortality, and the side-effect profile includes mostly transient and self-limiting adverse events. This likely safety and the beneficial effects of MSCs in other disorders in these trials (though variable), combined with the experimental indications of likely benefit in whole animal or cellular models have provided justification for clinical testing in MS. Initially, clinical trials focused on safety and proof-of-concept. Connick *et al* recruited ten participants with MS, and additional controls, in 2008-2009, and successfully isolated, expanded and characterised MSCs *in vitro*, which then lead to an open label safety and feasibility trial (7). An improvement in visual function was reported, as indeed had earlier been suggested in comparable studies by Yamout *et al* (56). Other similarly small trials have reported stabilisation of progression or a modest improvement in EDSS (Expanded Disability Status Score) with MSC infusion (see table 1). An international multi-centre trial, MESEMS (mesenchymal stem cells for multiple sclerosis) is currently ongoing (57).

At the same time, refinements of the MSC approach are already under experimental consideration: for example, priming cells in various ways in culture before infusion, or genetically modifying MSCs, in order putatively to improve aspects of their function including survival, neuroprotective or restorative function, or homing to specific target tissues. One example would be to increase the expression of hepatocyte growth factor, which has been implicated in the efficacy of MSCs in EAE (48).

Related approaches

MSCs can be obtained from tissues other than the bone marrow. 'PDA-001' is a preparation of mesenchymal-like cells derived from full-term human placenta tissue. PDA-001 caused a dose-dependent protection from EAE induction, and in established EAE, a reduction of disease progression and severity (58). PDA-001 has now also been investigated in a multi-centre, randomised, double-blinded trial in patients with RRMS and SPMS (59), the first therapeutic trial of its kind to investigate the human placenta as a source for therapeutic stem cells (Table 1). In this study, 81% of patients were taking at least one other licensed MS medication concomitantly, and so identifying treatment effect was complicated, but PDA-001 administration in patients with MS appeared to be both safe and feasible. PDA-001 may

have significant benefits as an alternative source of cells: the full-term placenta is a safe and plentiful source of non-embryonic cells; and production scalability is also feasible (59).

Within bone marrow, a number of stem cell sub-populations are present in addition to haematopoietic stem cells and MSCs. These include multipotent adult progenitor cells, and STRO-1-positive cells, both of which (and also including HSCs) have been reported to have reparative and neuroprotective properties. It is suggested that these various populations may contribute synergistically to promote tissue repair (3). Certainly, no one sub-population has been shown to be more effective than other sub-populations, and some studies report that the unselected (and unexpanded) mixed bone marrow mononuclear cell populations containing all these cell types and others may be more effective therapeutically than purified and expanded MSCs. The approach of utilising a filtered preparation of whole bone marrow, aiming to maximise the likelihood of including any and all sub-populations of potentially useful BM-resident stem cells, has been explored clinically in a number of disorders with apparent benefit. We have studied this approach in a small number of MS patients in an uncontrolled phase I trial (60). The data support the safety and feasibility of the approach as well as raising the possibility of a treatment effect. This therapeutic approach, were it to prove beneficial in larger controlled studies (61), would carry the additional advantage of practical ease of adoption and application in non-specialist units, lacking as it does in the cell expansion-related requirement for a Good Manufacturing Practice (GMP) cell culture and selection facility**.**

Neural stem or precursor cells (NPCs) also have neuroprotective properties, as shown in EAE models, where NPC transplantation can lead to significant reduction of the clinical severity of the disease and reduction of pathological parameters of inflammation (62). Using a viral model of demyelinating disease, intra-spinal transplantation of human embryonic stem cell-derived NPCs resulted in sustained clinical recovery (63). Clinical application of these cells in MS is being planned (61).

2: Efficacy and safety of trials in cell therapy

Table 1 shows an overview of reported trials of different types of cell therapy in MS. The great majority of these have explored AHSCT, with a mixed cohort of patients with MS, including those with RRMS or SPMS, so that distinguishing between efficacy for RRMS and for SPMS can be challenging. Still, given the likely substantial differences in mechanisms of tissue damage, and in clinical impact, it is worth attempting to explore the clinical trial data specifically for distinct effects on relapse activity and on progressive disease.

Efficacy – relapse suppression

Individual early case reports showed that treating patients with highly active RRMS using AHSCT was beneficial, particularly in cases with highly active inflammatory disease. These patients showed significant improvement in EDSS, and suppression of relapses over a period of 12-24 months, without any additional disease-modifying therapies (DMT). MRI findings also suggested no subclinical disease activity. These examples demonstrated the therapeutic potential of AHSCT (64, 65). Another case report explored the administration of Cy and non-myeloablative AHSCT (and ATG) in

a patient with malignant type MS, with a pre-treatment EDSS of 8.0, which improved to 6.5 after 1 year, with no new lesions demonstrated on MRI (66), again suggesting that AHSCT can be effective and safe even during periods of extreme inflammation and disability, with a lasting therapeutic effect. Similar dramatic improvements in EDSS with suppression of relapse activity have been noted in other patients with "malignant" RRMS (67). Recurrence of relapse after autologous HSCT can occur, however, and has been attributed both to the pre-transplantation conditioning regimen (68), with failure to eliminate all anti-myelin reactive cells, and also to the T lymphocytes that may be present among the autologous graft (69).

Following these earlier reports, Burt *et al* (70), utilising a non-myeloablative AHSCT approach in a relatively large study (123 patients with relapsing-remitting MS, and 28 with secondary-progressive disease), showed impressive outcomes, with 80% of patients showing relapse-free survival at 4 years. The adverse event profile was good, with a few cases of ITP and autoimmune thyroid disorder, and no transplant related mortality (TRM). It is worth noting that during the conditioning period, alemtuzumab was utilised, and since this immunomodulatory drug is highly effective in RRMS, it is difficult to isolate the benefit of AHSCT on its own.

Using a more aggressive immune-ablative approach, Atkins *et al* (71) recently reported dramatic relapse activity effects - with not a single relapse occurring in 24 patients post-AHSCT, and not a single gadolinium-enhancing lesion on repeated post-transplant MRI scanning. However, there were a number of adverse events, including hepatic necrosis (resulting in death), an ITU admission involving sinusoid obstruction syndrome, thyroid dysfunction, and febrile events including positive cultures.

Efficacy – preventing disability progression

Here, efficacy is often expressed as progression-free survival (PFS), defined as the absence of a confirmed increase in EDSS by at least 1 point. In studies with a follow-up of at least 2 years, progression-free survival ranged from 36% to 100%, and only a minority of patients showed an improvement in EDSS (22). There are a number of complications in assessing the clinical significance of such studies. First, disability progression in relatively short term studies in MS is notoriously unreliable, partly because progression is often very slow in MS, and partly because disability changes in relatively short term studies may substantially reflect improvement from pre-HSCT relapses rather than implying changes in underlying disease progression. Thus in Burman *et al*'s study, where improvement was reported, the majority of the improvement took place during the first year, with some additional improvement in the second year, but no further improvement subsequently (72). In the assessment of effects on disability progression, most authorities lend more weight to longer term studies, such as Fassas *et al* (73): here, progression-free survival was notably lower than in those studies with shorter term follow-ups.

Secondly, often impressive and sustained suppression of Gd+ lesions or overall volume of T2 lesion load reduction on MRI can been noted post-AHSCT (24, 74). Perhaps unsurprisingly, however, these positive MRI findings do not necessarily imply, and have not been accompanied by comparable improvements in clinical disability in patients. Suppression of MRI enhancement in trials using myeloablative regimens in patients with progressive disease is difficult to interpret, as in the progressive phase of MS, MRI enhancement normally decreases spontaneously (75).

And thirdly, the relationship between relapses and disease duration prior to treatment should be considered; relapse frequency decreases with disease duration in MS, and so the results of studies assessing disability progression are less likely to be 'contaminated' by relapses (and recovery) if patients with more chronic disease are targeted. Burt *et al* found that the EDSS score did not improve in patients with disease duration longer than 10 years (70) (or more generally in patients with SPMS). A related, confusing influence of relapses may explain the results of studies showing that patients with SPMS have a higher probability of remaining 'progression-free' than those with PPMS (68).

In a retrospective survey of the EBMT database, the advantages of treating early, in the inflammatory phase of the disease are discussed: younger patients, transplanted within 5 years from diagnosis, showed significantly better progression-free survival (19). Similarly, Krasulova *et al* commented that patients with relapsing MS, disease duration <5 years and age <35 years old have a more favourable outcome from AHSCT (76). MS patients with long-lasting disability have been shown to be poor responders to HSCT, presumably due to the likely irreversibility of chronic lesions (69).

In Burt *et al*'s study exploring less intense immunosuppression (68), 87% of patients were found to have progression-free survival. It is worth mentioning that the mean age of patients in this trial is lower, and mostly RRMS patients were recruited. The trial also had a relatively short follow-up period (median follow-up 2 years). Even with more intense myeloablation, Atkins reported 69.6% of patients to have disease-free survival at 3 years (71).

Safety

The majority of trials have explored conditioning regimes utilising BEAM therapy – carmustine (**B**iCNU®), **E**toposide, cytarabine (**A**raC) and **M**elphalan – combined with mobilising procedures that include cyclophosphamide (Cy) and G-CSF, CD34+ selection and ATG *in vivo* purging. The cytotoxic agents involved carry significant potential side effects, and immunoablation naturally also carries significant risks: hence the need seriously to consider the adverse effect profile of AHSCT.

Myeloablative transplant regimens (such as total body irradiation (TBI) or full-dose busulfan) cause irreversible bone marrow failure, thus absolutely requiring haematopoietic stem cell re-infusion to regenerate bone marrow function. Toxicity and late complications can be substantial with myeloablative regimens (70), as demonstrated in Table 1. TBI is associated with a higher mortality. It has also been speculated that TBI may induce an endogenous factor that enhances demyelination or interferes with ongoing remyelination (24). The disadvantage of adding Cy to G-CSF is the increased risk to the patient due to an additional pancytopenic interval, increased cost of management of patients receiving chemotherapy, and the delay in proceeding to high dose immunosuppressive therapy (68). Fassas *et al*'s study (4) had a high mortality, but did valuably demonstrate that there was no evidence that more intense conditioning, purging or ATG use was associated with higher probabilities of confirmed progression-free survival (4).

The most frequent adverse event noted in AHSCT was febrile neutropenia. There is also a high incidence of urinary tract infection, which is to be expected in patients with MS, who often already have neurological problems of bladder dysfunction, particularly in the progressive phase of the disease. The increased risk of infections in patients with reduced

mobility, together with restrictive pulmonary defects, supports the current suggestion of including patients with lower EDSS scores (22). The most frequent late adverse events reported in MS patients undergoing HSCT are varicella-zoster virus and herpes simplex virus reactivation, followed by the development of autoimmune diseases, including autoimmune thyroiditis (22).

Transplant-related mortality (TRM) is plainly the greatest concern: any risk might be considered too high in relation to a condition – MS – which is not life-threatening *per se* (77). One retrospective survey looked at 183 patients with MS in the database of the EBMT Registry (19). The overall TRM was 5.3%, but importantly this mortality was noted only in the period of 1995-2000, with an apparent 0% TRM reported subsequent to 2000. Also, no deaths were noted in those treated with BEAM without graft manipulation. Improvement or stabilisation of neurological condition was noted in 63% of patients, at a median follow-up of 41.7 months, and was irrespective of the conditioning regime. The analysis also suggested that in those using a moderate conditioning regime, a durable benefit was seen in some patients, quoting figures post-HSCT of up-to nine years (19). These observations provided further impetus for exploring alternative approaches to conditioning, although it should also be stressed that better patient selection criteria, and better supportive care, including infection prophylaxis are also likely to have contributed to the more recent reduction in TRM.

Hamerschlak *et al*'s study is the only trial that has directly compared the toxicity of different conditioning regimes (78) – BEAM/ATG (horse) against the Cy/ATG (rabbit) regimen. The overall complication rate in the BEAM/ATG group was 71.4% - considerably higher than the Cy/ATG group figure of 40%. Three subjects (7.5%) died (of cardiac toxicity, sepsis and alveolar haemorrhage), all of them in the BEAM/ATG group. Moreover (and as with the retrospective EBMT Registry survey), the efficacy results were broadly similar, although the period of follow-up was relatively short (78).

Cost-benefit and risk benefit

Measurement of long term benefit in MS clinical trials has long been recognised to be extremely challenging. Determination of the risk-benefit ratio is also difficult, especially for patients with early MS, with mild to moderate disability and low EDSS scores, since the prognosis for long term survival is good, despite worsening physical ability (79). Six years ago, and in the most optimistic scenario, the cost-effectiveness of AHSCT was considered to be around £2800 per additional quality-adjusted life year (QALY) gained (5). The initial costs of HSCT are extremely high; and for any new and costly treatments to be applied widely in a resource-constrained health service, such as the National Health Service in the U.K., and many other health services, it is necessary to demonstrate value for money in the context of other competing priorities (5). At present, there have been no phase III prospective randomised studies that compare the efficacy of AHSCT against other conventional therapies. The only comparative trial is the Autologous Haematopoietic Stem Cell Transplantation trial in MS (ASTIMS), a phase II study that was designed to assess the effect of AHSCT vs. mitoxantrone (MTX) on disease activity in MS, measured by MRI in the 4 years following treatment (80). The results of this trial are summarised in table 1. In terms of cost-effectiveness and benefit of AHSCT, considering a 6-month sustained progression rule, the study demonstrated that AHSCT is less effective than mitoxantrone, using a decision-analytic Markov model for evaluation (80). Mitoxantrone is little used now in MS, diminishing the practical value of this study.

To assess the risk-benefit ratio of HSCT in MS, Daumer *et al* investigated the natural history of moderately severe MS, and concluded that the probability of reaching an EDSS score of 10 (death), after 15 years was 22%. In Fassas *et al*'s study, exploring the long term outcome of HSCT, the combined disease-related mortality and procedure-related mortality was 17%, thus at face value comparatively favourable (73, 79). In Daumer *et al*'s study, the risk for progression to advanced disability, defined as an EDSS score of 8, was very low for the subgroup with a baseline EDSS score of 3-3.5; however, for those with a baseline EDSS score of 4-5.5, 3% had advanced disability after two years, 5% after three years, 6% after four years, 12% after five years, and 40% after 10 years (79). In light of this, the progress-free survival rates of AHSCT trials might be seen as favourable, although there is little evidence from long term follow-up studies.

In summary, there are clearly still significant gaps in the evidence, and the next steps would involve exploring phase III randomised trials, with larger recruitment of patients and longer follow-up, and in particular with comparison against current licensed more potent treatments, including natalizumab and alemtuzumab, to elicit the true efficacy of cell therapy, and to assess the cost-effectiveness and risk versus benefit quotient in these patients. Only one trial with considerable follow-up of 11.3 years commented that disease progression (with or without initial improvement post HSCT) still occurred in a significant proportion of their patients despite impressive sustained effect in suppressing activity on MRI, suggesting that HSCT is not a therapy for the progressive population of MS, and should be reserved for those with aggressive relapsing disease, in the inflammatory phase and for the malignant form of MS (67, 73).

Conclusion and future considerations

Considerable advances in our understanding of MS physiology have allowed a paradigm shift in the management of MS from one that simply targets CNS inflammation towards one that at least aims to be both immunomodulatory and neuroprotective, and which additionally carries the potential for regenerative repair. Cell therapies intended to achieve repair by direct cell replacement have made limited progress towards clinical application, largely because of questions concerning the basis of this approach; however, related studies of the cellular biology of remyelination have yielded a number of molecular candidates for more conventional pharmacological approaches to myelin repair.

Concerning HSCT, better outcomes are evident in patients with active inflammatory disease, shorter disease duration, and lower EDSS scores; and in those with RRMS rather than SPMS and PPMS. This is consistent with a treatment targeting control of peripheral immunity rather than directly affecting pathological processes within the CNS (22). The increasing experience of neurologists and haematologists with conditioning regimes, with myeloablative versus non-myeloablative treatment protocols, and in the management of adverse effects, has led to significant reductions in TRM. While the precise place of HSCT in the overall treatment paradigm for MS remains to be defined, it is increasingly no longer seen as a last resort for patients with a poor prognosis (22).

Recent trials are exploiting the immunomodulatory, neuroprotective and reparative properties of other bone marrow-derived stem cells, such as MSCs, and of comparable cells from other sources. These approaches carry a number of practical advantages, including relative ease of access and safety of administration, as well as avoiding the need for immunosuppressive treatment to prevent rejection (41). Thus far, published trials have been limited to small safety and feasibility studies, and while these have shown a favourable adverse event profile, the efficacy of MSC transplantation has been modest. The same applies to trials that have explored the avenue of non-selected, non-expanded cells. Phase II/III trials of both approaches are now underway (57, 61, 81). With regards to other cell types, such as human placental-derived stem cells, there is a considerably greater sparsity of trial evidence (59).

In an era where cell therapy has been rapidly expanding in other fields such as cardiovascular medicine, and with the limited options of conventional treatments available for progressive MS, there is a drive to accelerate trials in MS to explore the efficacy and cost-effectiveness of cell therapy. However, it is only by recruiting patients to carefully designed clinical trials and populating detailed registries, that we will acquire data to enable us to answer the question of whether cell therapy is truly beneficial to the general population of patients with MS.

Table 1: Overview of clinical trials of cell therapy in MS

Part A: Summary of HSCT/BMT trials

Author	Time-frame	Type of MS (number of patients or %)	Age	Follow-up (years) mean/ median	Treatment	Outcomes	Adverse Events (number of patients or %)
Fassas et al (73)	1995-2001	SPMS (19) PRMS (4) RRMS (1) PPMS (1)	Median = 40 Range = 9-54	11.3	Cy+G-CSF, BEAM or busulfan +ATG, IV PBSC	-EDSS improved 26% -11.3 year PFS 25% -Gd+ lesions reduced from 9.53cm^3 to 0.17cm^3	-TRM (2) (aspergillosis and pulmonary haemorrhage)
Nash et al (68)	1998-2001	PPMS (14) SPMS (17) RRMS (1)	Median = 41	2	Cy+G-CSF, TBI+ATG, IV PBSC	-Progression estimate 3 years 27% -Gd+ lesion volume decrease- 6.6% (1 year)	-TRM (1)-EBV PTLD -Engraftment syndrome (13/18), MS flare (1), irreversible neurological deterioration (1), UTI (8), bacteraemia (4), central venous catheter infection (1), viral self-limiting illness (7), ITP (1), brachial neuritis (1)
Mancardi et al (18)	1998	SPMS (10)	Median = 35.5 Range= 26-52	1.25	Cy+ G-CSF, BEAM, IV PBSC	-Gd+ suppression 100% -EDSS 6.5 to 6.0 (6 months)	-No TRM -Febrile neutropenia (9), transient elevation of liver enzymes (2), rash (1), UTI (3), non- or symptomatic CMV reactivation (3), gastric pain (1), subclavian phlebitis (1), SIADH (1)
Capello et al(82)	1998	RRMS (4) SPMS (17)	Median =24	2	Cy+G-CSF, BEAM +ATG, IV PBSC	-EDSS stable or improved 95%	Haemorrhagic cystitis (1), subclavian phlebitis (1), transient SIADH (1), CMV reactivation (6)
Saccardi et al (83)	1998-2003	SPMS (15) RRMS (4)	Median =36 Range= 26-52	3	Cy+G-CSF, BEAM, +ATG, IV PBSC	-Gd+ suppression 95% -6 year PFS 95% -4.5 year DFS 64%	-No TRM -Fever (16), haemorrhagic cystitis (1), UTI (1), CVC-related phlebitis (1), inappropriate secretion of ADH

							(1), sepsis (5), enteritis (1), transient elevation of LFTs (1), gastric ulcer bleeding (1), HZV infection (1), transient monoclonal gammopathy (1)
Kozak et al (84)	1998-1999	SPMS (11)	Range =25-44	0.71	Cy+G-CSF, BEAM+/-ATG, IV PBSC	-EDSS improvement 77%, -Gd+ suppression 55%	-No TRM Febrile neutropenia (all), Gram + bacteraemia (2), arm cellulitis (1), herpes zoster (1)
Fassas et al (4)	1999-2000	PPMS (26%) SPMS (70%) RRMS (4%)	Median =39 Range= 20-58	1.3	Cy +/-G-CSF, BEAM, Cy +/-ATG or TBI and busulphan, IV PBSC and BM	-EDSS improvement by ≥1 in 21% at 3 years -PFS 74% -Disease progression in 20% -Gd+ lesions in 33% pre- to 8% post-transplant	-TRM – 7 patients (5 cytotoxicity, 2 neurological complication) -Neurological deterioration (27%), infection/allergic events/severe G-CSF induced bone pain (15%), infection/cardiac and hepatic toxicity, bleeding, TTP (59%)
Shevchenko et al (16)	1999-2006	SPMS (27) PRMS (1) PPMS (11) RRMS (11)	Median = 32 Range =18-51	1.6	Cy + G-CSF BEAM+ ATG, IV PBSC	-EDSS score of 0.5-62.5% at 1.6 years -Gd+ lesions suppressed 43.3% -6 year PFS 72%	-No TRM -Neutropenic fever (51.6%), hepatic toxicity (48.1%), transient neurological dysfunction (22.2%), enteropathy (18.5%), sepsis (2%)
Krasulova et al (76)	1999-2008	RRMS (11) SPMS (15)	Median =33	5.5	Cy+G-CSF, BEAM+ T-cell depletion, IV PBSC	-PFS estimate 70.8% at 3 years, 29.2% at 6 years	-No TRM -Febrile neutropenia (14), sepsis (11), UTI (7), diarrhoea (16), mucositis (11), arthralgia (1), HSV1 and VZV (1), chronic hep B (1), GBM (1), Acquired anti-factor VIII inhibitor (1)
Ni et al(69)	2000-2005	SPMS (16) PPMS (2)	Median = 37	3.5	Cy+G-CSF, TBI or BEAM	-3.5 year PFS 75% -3.5 year DFS 33.3%	-TRM (2)- severe pneumonia and

		PRMS (2) Malignant MS (1)	Range= 15-58		+ATG, IV PBSC	-Gd+ lesions post-transplant 14.3%	varicella-zoster virus hepatitis -Allergy (4),infection (8), elevation of liver enzymes (6), transient neurological deterioration (5), depression (5)
Chen et al (14)	2000-2007	SPMS (19) PPMS (1) RRMS(3) PRMS (2)	Median = 37.3 Range = 15-64	4.9	Cy+G-CSF, BEAM+ ATG, IV autologous PBSC	-EDSS 8.0 to 5.5-7.0 (1 year) -3 year PFS = 74% -Gd+ lesions suppressed/nil new 58%	-TRM (2)-Pneumonia (1) Varicella-zoster virus hepatitis (1) -Bacterial infection (13)
Opensha w et al (24)	2000	SPMS (5)	Range = 39-47	2	G-CSF, Busulfan+ Cy+ATG, IV PBSC	-2 year EDSS improvement 50%, - Gd+ suppression 100%	-TRM (1)-influenza A pneumonia -Line infection (1), *C.diff* diarrhoea (1), severe MS flare (1) -NTRM-*S.pneumonia* sepsis
Hamers chlak et al (78)	2001-2006	PPMS (4) SPMS (33) RRMS (4)	Mean = 42 Range = 27-53	1.5	Cy+G-CSF then BEAM/ ATG (horse) or CY/ATG (rabbit), IV PBSC	-EDSS improved 63.2% (no difference between two regimes) -No new Gd+ lesions	-TRM 3 in BEAM/ATG group (cardiac toxicity/sepsis/alveolar haemorrhage) -Febrile neutropenia (18), pneumonia (8), allergy to ATG (5), UTI (7), DVT and PE (3), depression (3)
Atkins et al (71)	2001-2009	RRMS (12) SPMS (12)	Median =34 Range= 24-45	6.7	Cy+G-CSF, Busulfan, Cy+ATG, IV PBSC	-3 year DFS 69.6% -No new Gd+ lesions	-TRM – hepatic necrosis (1) -UTI (13%), ITU admission (sinusoid obstruction syndrome), febrile neutropenia (all), positive cultures (29), viral infections (26%), thyroid dysfunction (5), immune thrombocytopenia (1)
Burt et al (85)	2003	RRMS (21)	Range = 21-52	2.6	G-CSF+Cy, IV PBSC	-EDSS ≤6.0 stable 43% -Gd+ suppression 57%	-TRM (2), -Pseudomonas bacteraemia (1), dermatomal zoster (2), disseminated zoster (1),

							rash/fever/fatigue (5)
Burt et al (75)	2003-2005	RRMS (21)	Range =20-53 Median =33	3.1	Cy+G-CSF, Cy+ Alemtumuzab/ATG, IV PBSC	-EDSS improvement 1 point 81% -3 year RFS 76% -3 year DFS 62%	-No TRM -*C.diff* diarrhoea (1), dermatomal zoster (2), ITP (2), neutropenic fever (5), transient neurological hypoaesthesia (1)
Burt et al (70)	2003-2014	RRMS (123) SPMS (28)	Mean = 36 Range = 18-60	2.5	Cy+G-CSF, alemtuzumab /ATG, IV autologous PBSC	-EDSS 4.0 to 2.5 (4 years) -4 year RFS = 80% -4 year PFS= 87%. -T2 lesion volume 8.57cm^3 to 5.74cm^3 (27months)	-No TRM -Dermatomal zoster (4), ITP (7), hypothyroidism (7)
Saiz et al (86)	2004	SPMS (9) RRMS (5)	Median = 30 Range= 22-45	3	Cy + G-CSF, BEAM+ ATG, IV PBSC	-3 year PFS 85.7% -3 year DFS 46.4% -No new T1 lesions -50% reduction in T2 lesion volume	-No TRM -Neurological deterioration (3), secondary amenorrhea (4)
Fagius et al(67)	2004	RRMS (9)	Median = 27 Range= 9-34	2.4	Cy+G-CSF, BEAM+ ATG, IV PBSC	-EDSS improvement 3.5 -No new T2 lesions	-No TRM -Crohn's disease (1) -Mucositis, alopecia, sepsis (2), serum sickness (2), herpes zoster (1)
Mancardi et al (80)	2004-2009	SPMS (6) RRMS (7) PRMS (8)	Mean =36, Range = 22-46	4	Cy + G-CSF, BEAM+ ATG and IV PBSC, compared with mitoxantrone (MTX)	-AHSCT reduced number of T2 lesions by 79% compared to MTX. -No difference noted in the progression of disability	-No TRM -Febrile neutropenia/diarrhoea/leukopenia/mucositis/anaemia/amenorrhea, reduced platelet count (80%) -Prolonged hospitalisation with late engraftment (1), systemic candidiasis and CMV reaction (1), ATG reaction (1)
Bowen et al (87)	2005-2008	SPMS (17) PPMS (8) RRMS (1)	Median = 41 Range = 27-60	4	TBI, Cy + ATG, IV PBSC	-EDSS improved 15% -3 year PFS 63% -6 year PFS 48% -T2 lesion volume decrease 12.3% (3 years)	-TRM- EBV PTLD (1) -NTRM (4) -Myelodysplastic syndrome (post 7 years- Tx with mitoxantrone)
Shevchenko et al (88)	2005-2011	RRMS (43) SPMS (56)	Mean = 35	4	BEAM-like conditioning, G-CSF, IV PBSC	-8 year disease progression 16.7% -Event free survival 80%	No TRM

Shevchenko et al (23)	2006-2011	SPMS (35) PPMS (15) PRMS (3) RRMS (42)	Not given	3.8	G-CSF, BCNU/ CCNU and melphalan/ mini-BEAM like+ ATG, IV PBSC	-EDSS improvement or stabilisation 80% -5 year PFS 92% (early AHSCT), 73% for conventional salvage AHSCT	-No TRM -Thrombocytopenia (100%), neutropenia (100%), fatigue (100%), anaemia 80%), alopecia (80%), hepatic toxicity (42.1%), transient neurological decline (27.4%), enteropathy (7.4%), skin allergy (8.4%), pneumonia (2.1%), uterine bleeding (2.1%), oral herpes (1.05%), genital, herpes (1.05%), sepsis (3.2%)
Xu et al (89)	2001-2006	SPMS (22)	Median =35.5 Range= 20-51	3.25	G-CSF, BEAM, IV PBSC	-3.25 year PFS 77% -59% of patients had neurological improvement	-No TRM -Diarrhoea (13), fever (6), transient neurological decline (8), bacterial infection (7)
Samijn et al (90)	2006	SPMS (14)	Median =35 Range= 23-50	3	Cy, TBI+ATG, IV BMSC	-EDSS improved 14% -3 year PFS 36% -No new Gd+ lesions	-No TRM -Mucositis (10), rash (6), alopecia (all), fatigue (all), *C.diff* diarrhoea (2), fever (all), EBV PTLD (1), anti-thyroid antibodies (3), myelodysplastic syndrome (1), herpes zoster (1), neurological deterioration (2), muscle spasms (2), loss of visual acuity (3)
Rocca et al (28)	2007	SPMS (14)	Mean = 38 Range = 23-50	3	ATG+Cy, TBI, IV PBSC	-Gd+ suppression - 100% -Stabilisation or improvement in EDSS in 35.7%	-No TRM -EBV PTLD (1), anti-thyroid antibodies (3), myelodysplastic syndrome (1)
Burman et al (72)	2014	RRMS (40) SPMS (5) PPMS (5)	Mean =31 Range= 9-52	3.9	Cy + GC-CSF, BEAM+ ATG or Cy/ATG, IV PBSC	-5 year relapse free survival 87% -MRI event-free survival 85% -5 year PFS 77% -5 year DFS 68%.	-No TRM -Herpes zoster reactivation (15%), thyroid disease (8.4%), neutropenia fever (17), invasive fungal infection (1),

							Crohn's disease (1), alopecia areata (1), epilepsy (1)

Part B: Summary of MSC/related trials

Author	Time-frame	Type of MS (number of patients or %)	Age	Follow-up (years) mean/ median	Treatment	Outcomes	Adverse Events (number of patients or %)
Rice et al (60)	2007-2009	RPMS (6)	Mean = 47.7	1	IV BMSC	-Multi-modal evoked potentials showed significant neurophysiological improvement	Transient increase in lower limb spasticity (2), urinary retention (1)
Connick et al (7)	2007-2010	SPMS (10)	Mean = 48.8, Range = 40-53	18 months	IV (BM) autologous MSC	-Improvement in visual acuity and visual evoked response latency -Increase in optic nerve area	Rash following infusion (all), Bacterial infection (2)
Llufriu et al(91)	2010-2012	RRMS (5)	Median age = 41 Range= 23-48	1	IV (BM) autologous MSCs	-Gd+ lesions at 6 reduced (12.3 to 3.1)	Upper respiratory infection (1), influenza (1), gastroenteritis (1), herpes labialis (1)
Karussis et al (41)	2010	SPMS (17) PPMS (8) RRMS (1)	Mean = 35.3 Range = 27-60	2.1	IT (BM) autologous MSCs	-EDSS 6.7 to 5.9 -No new Gd+ lesions at 6 months	-No TRM -Transient fever (21), headache (15), meningeal irritation and aseptic meningitis (1)
Yamout et al (56)	2010	SPMS (9) RRMS (1)	Range =34-56	1	IT (BM) autologous MSCs	-Vision and low contrast sensitivity at 3 months improved in 83%	Transient encephalopathy (1), cervical and back pain (1)
Bonab et al(92)	2008-2010	SPMS (23) PRMS (2)	Mean= 34.7	1	IT (BM) autologous MSCs	-EDSS 6.1 to 6.3 (1 year). -72.8% EDSS stable - Gd+ lesions post-transplant 25%	-No TRM -Low-grade fever (all), nausea-vomiting (2), weakness in lower limbs (2) and headache (3)
Lublin et al (59)	2010-2011	RRMS (10) SPMS (6)	Low dose median 52.5, high dose median 47.5	1	IV Human placenta tissue (non-autologous), PDA-001 (mesenchymal-like stem cells)	-Stable or decrease in EDSS 94%	-No TRM -MS flare (6%), anaphylactoid reaction (6%), superficial thrombophlebitis (6%), headache (44%), URTI (31%), fatigue

							(25%), infusion site reactions/events (4) and UTI (25%)
Li et al (93)	2010-2012	23 (RRMS+ SPMS), 10 given placebo	Mean= 41.7	1	IV non-autologous Human umbilical cord-derived MSCs	-EDSS score and relapse recurrence significantly lower than the control group	None reported
Cohen et al(94)	2014	RRMS (10) SPMS (14)	Mean = 46.5	0.5	IV autologous MSCs with human fibroblast growth factor 2	-No significant improvement noted	No serious AE reported

PBSC=peripheral blood stem cells, BMSC=bone-marrow derived stem cells, BM=bone marrow, TRM= treatment related mortality, PFS=progressive free survival, RFS= relapse free survival, DFS=disease activity free survival, MSC=mesenchymal stromal cells, Gd+=Gadolinium enhancing MRI lesions, Cy=cyclophosphamide, G-CSF=granulocyte colony stimulating factor, BEAM=carmustine, etoposide, cytosine-arabinoside, melphalan, TBI=total body irradiation, ATG=anti-thymocyte globulin, IT=intrathecal, IV=intravenous, EBV PTLD=Epstein Barr Virus Post-Transplant Lymphoproliferative Disorder, Tx=treatment. Unless otherwise stated, clinical outcome data is stated for the end of the follow-up period.

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